Comparative ultrastructural investigations of the podocyte in marine bivalve *Pinctada radiata* (Pteriidae) and freshwater bivalve *Anodonta rubens* (Unionidae)

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Abstract Ultrastructure of the podocyte of the pericardial gland in marine bivalve *Pinctada radiata* and in fresh water bivalve *Anodonta rubens* were investigated. Obvious differences in shape, distribution and number of cytoplasmic organelles of the podocytes observed in both species are suggested to be related to the difference of their habitats. In addition, the pedicels of the basal membrane are more complex in *A. rubens* than those in *P. radiata*, and also the surface of the apical cell differs in structure. The suitability of podocyte structure to its function is discussed.

Key words: bivalve, excretory system, pericardial gland, podocyte, ultrastructure

Introduction

The anatomy and histology of the bivalve excretory system have been comprehensively given by White (1942) and Narain (1976). However, most of the recent studies on the excretory system were concerned with only the pericardial gland, which is considered the site of ultrafiltration. Kato (1960) described the structure of this gland in seven species of lamellibranchs and studied its role in excretion. Moore et al. (1980) studied the cytology and cytochemistry of the gland of *Mytilus edulis*. Morse (1990) demonstrated a kind of muscle network in the pericardial gland of *Chlamys hastata* and suggested its possible role in ultrafiltration. Some further investigations on ultrafiltration of excretory system have been done. Pirie and George (1979) started these works on *Mytilus edulis*, then Jenning (1984) on some estuarine and freshwater bivalves. Recently, Andrews and Jennings (1993) postulated its notable role in urine formation of some bivalves from various habitats. Other authors were interested in the urine formation, excretion and osmoregulation in both marine and freshwater bivalves. Potts (1954) and Peggs (1975) demonstrated the urine formation and excretory ultraformation of *Anodonta cygnea*. Deaton et al. (1989) and Deaton (1992) studied the osmoregulation and epithelial permeability in some bivalves.

In recent years studies on excretory system enter the applied scale. Hemelraad et al. (1990) investigated the ultrastructural changes in the renal system of different species of bivalves under the effect of cadmium. Cockcroft (1990) made a comparative study on the nitrogenous excretion of two species of *Donax* sp., and Wohlgeschaffen et al. (1992) followed the excretion of phycotoxin domic acid in *Mytilus edulis* and *Placopecten magellanicus*. Special attention has been paid to podocytes because they are the sites for ultrafiltration with basely extending pedicels forming an interdigitating network apposed to basal lamina. All podocytes have electron-dense granules, Golgi complex and vacuoles distributed in their cytoplasm. Piere and George (1979) and Jennings (1984) have described the presence of podocytes within the pericardial glands of several bivalves by electron microscopy.

As the present two investigated species live in different aquatic media; it is suspected that there must be differences in the structure of the excretory system to adapt the respective habitats. Therefore a comparative investigations of the excretory system of these species

will supply us with fruitful knowledge in this concern. In the present paper, special attention is paid to the fine structure of the excretory unit and the podocyte of the pericardial gland in order to reveal the difference and adaptation for the change of the habitats.

Materials and Methods

Pinctada radiata (Leach, 1814) were collected from Abu Kir Bay, Alexandria in the Mediterranean Sea from between rocks. *Anodonta rubens* (Linnaeus, 1758) were collected from the River Nile's branch in El-Gharbia province where they live burrowing in mud.

Small pieces of pericardial gland of both species were fixed in 3% glutaraldehyde in 0.1M phosphate buffer (pH 7.2–7.4) for two hours at 4°C, washed in the same buffer for two hours and post-fixed in 1% osmium tetra-oxide. Fixed pieces were washed again by the same buffer for two hours at 4°C, then dehydrated rapidly through ascending grades of ethanol and embedded in Epon 812. Semithin sections for light microscopy were double-stained with 1% toluidine blue. For TEM ultrathin sections were cut on LKB ultramicrotome, double-stained with uranyl acetate, and lead citrate and then examined with a JEOL 100CX.

Abbreviations used in the Figures

AP: apical papilla, Au: auricle, BL: basal lamina, CF: collagenous fibers, Ci: cilia, CP: coated pit, D: desmosome, DK: distal portion of kidney, ER: endoplasmic reticulum, EP: excretory pore, EV: endocytotic vesicle, G: granules, GA: Golgi apparatus, IS: intercellular space, K: kidney, L: lumen, Ly: lysosome, M: mitochondria, MF: muscle fiber, N: nucleus, Ne: Nebenhole, NM: nuclear membrane, Nu: nucleolus, P: podocyte, PAM: posterior adductor muscle, PC: pericardium, Pd: pedicel, PG: pericardial gland, PK: proximal portion of kidney, PV: pinocytotic vesicle, R: rectum, RER: rough endoplasmic reticulum, RPC: renopericardial canal, SER: smooth endoplasmic reticulum, US: urinary space, V: vacuole, Ve: ventricle.

Results

1. *Pinctada radiata*

The pericardial gland is a diverticulum of the auricle with a central lumen and a deeply convoluted wall (Fig. 1). The lumen of the pericardial gland is lined with a layer of long columnar epithelial cells. These cells are laying on a basal membrane and are limited by regular plasma membrane (Pl. I,1). Cytoplasm of the podocytes contains numerous mitochondria, small Golgi vesicles, few units of endoplasmic reticulum, a large number of secretory granules which vary in their electron density, size and shape, and many different types of lysosomes (Pl.I,1). Each cell possesses a large oval nucleus with some small lateral nucleolus and homogeneously scattered chromatin materials. Each nucleus is limited by regular nuclear membrane. Under this membrane, a homogeneously distributed heterochromatin layer was seen (Pl. I, 1,4; Pl. II, 5). The apical surface of the podocyte is ciliated (Pl. I,2). Each cilium has a basal granule embedded in the cell cytoplasm, and a constriction gives rise to the shaft which is provided with the essential motile elements (Pl. I,3). The cell bodies are separated by large intercellular spaces that communicate with the pericardial cavity (Pl. I,2,4), thus the lateral cell walls are detached and connected together by the desmosome junctions which are clearly observed only near the apical ends of the cells (Pl. I,3) At the apical surfaces and in adhering area of adjacent podocytes, there are many cytoplasmic projections extending towards the pericardial lumen (Pl. I,3). Coated endocytotic vesicles are noted in the apical cytoplasm beneath the formed coated pits in the apical surface of the podocyte membrane (Pl. I,3). The mitochondria, which are circular to oval in shape with few crista, are distributed in the apical portion of the cell and around the nucleus (Pl. I,2; Pl. II,7). The cytoplasmic granules and vesicles

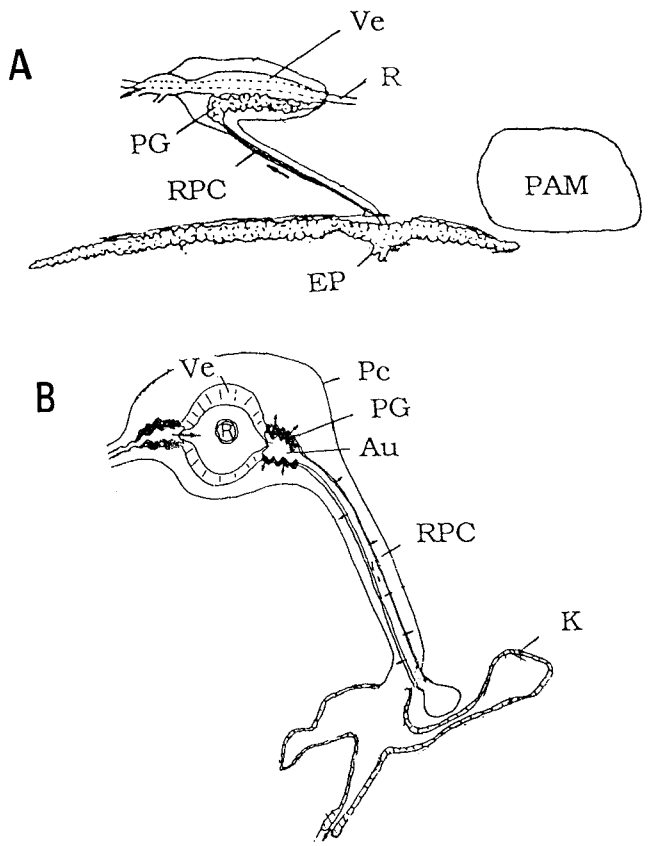


Fig. 1. Schematic representation of the excretory system of *P. radiata*, (A) lateral view and (B) transverse section.

are referred to as electron-dense granules (Pl. I, 1; Pl. II, 6). They vary in shape, size and content density (Pl. II, 6). Small Golgi units are found in close association with the primary lysosomes normally present in the cytoplasm (Pl. II,8). The secondary lysosomes are seen as membrane bound bodies scattered in the cytoplasm containing variable secretory electron-dense granules. The vesicles containing few particles of secretory granules are considered to be the residual bodies (Pl. II,9). Throughout the cytoplasm, small units of endoplasmic reticulum are seen as scattered granular vesicles or short smooth tubules (Pl. II,5,8).

The basal cytoplasmic membrane of the podocyte branches off into numerous cytoplasmic extensions, which terminate at the basal lamina (Pl. I,1; Pl. II,9). These processes or diaphragms are referred to as pedicels. They form a network lead to urinary spaces that connect with the intercellular spaces (Pl. II,9; Pl. III,11).

Large multi-vesicular bodies or endocytotic vacuoles are seen in the basal cytoplasmic portion of some podocytes (Pl. I,1). These vacuoles contain various microvesicles with various electron-dense inclusions and dense secretory granules (Pl. III,10). The basal lamina is a layer of fine fibrous structure adjacent to connective tissue and muscle cells (Pl. III,10,11).

2. *Anodonta rubens*

The pericardial gland is an outgrowth of the pericardium composed of numerous twisted

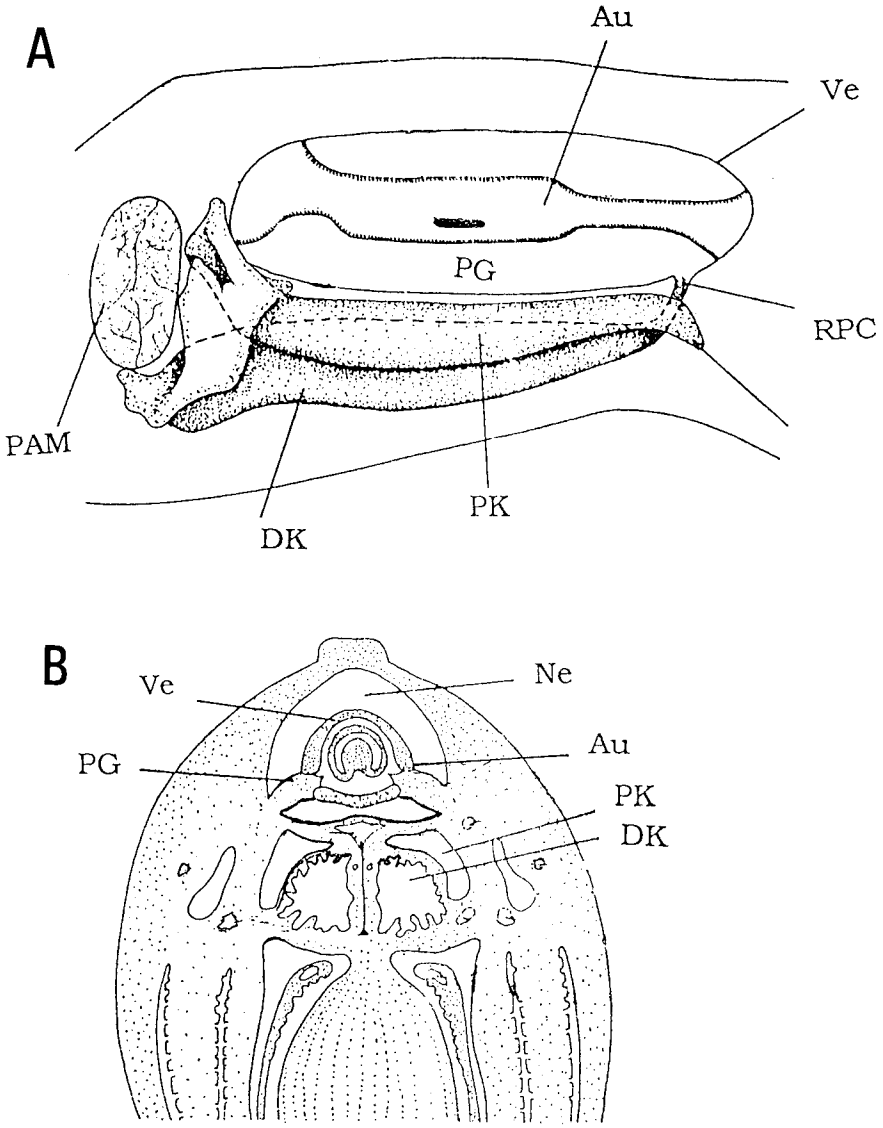


Fig. 2. Schematic representation of the excretory system of *A. rubens* as (A) lateral view and (B) transverse section.

tubules (Fig. 2). The wall of the pericardial gland is formed of epithelial cells varying in shape and size due to folds in the epithelium. The shape of the cell varies from cylindrical to pyramidal ones (Pl. IV,2). The cytoplasm of these cells is more electron-dense than that of *P. radiata*. The cytoplasm regularly extends from the podocyte cell body. These units branch off to form the interdigitating pedicel network opposing the basal lamina. This arrangement results in more regular urinary spaces that eventually communicate with the pericardial cavity (Pl. V, 6).

The apical surfaces of the podocytes bear numerous cilia shorter than those of *P. radiata* (Pl. IV,1,2). The undulating apical membrane of some cells gives off cytoplasmic projections

called apocrine papillae. Each papilla consists of a short swollen distal stalk (Pl. IV,1,2). This papilla leads to exocytosis. The intercellular spaces between the podocytes are regular, and narrower than those in *P. radiata.* Cellular junctions between the adjacent cells are seen (Pl. V,4).

The cytoplasm of podocytes has a large number of oval and elongated mitochondria, few Golgi vesicles, and elongated units of endoplasmic reticulum, which lie near the nuclei (Pl. V,3,5). Many lysosomes are seen in the cytoplasm forming a vascular system of various shapes and sizes of vesicles which are empty, or filled with electron-dense materials (Pl. V,3,5). Large membrane-bound vacuoles are seen in the basal portion of cytoplasm of some podocytes with different inclusions and structures (Pl. V,5).

Each podocyte cell has irregular shaped nucleus with some central nucleoli and dense large chromatin particles. The nucleus is limited by irregular membrane. A homogeneously distributed heterochromatin layer was seen under the membrane (Pl. V, 3,4).

The basal membrane of the cell branches off to form extensive interdigitating pedicel network that is more complex than that of *P. radiata* (Pl. V, 6).

Discussion

The podocytes, the characteristic cell type associated with ultrafiltration or primary urine formation, are observed in the excretory organs of both investigated species. But they have some differences in distribution, i.e. in the freshwater *Anodonta rubens*, podocytes exist in the walls of the auricles, the pericardial wall lining the cavity (Nebenhole), the pericardial glands, and the distal portion of the kidneys, while in marine *Pinctada radiata*, they do only in the pericardial glands. This difference may be related to the difference in habitats between the two species, which is surely related to osmoregulation mechanism. In *P. radiata* the pericardial gland is the only site of ultrafilteration where it gives a limited flow of filterate appropriate for living in marine habitat, while in *A. rubens* the distribution of podocytes along the excretory organs gives a much wider range of flow to avoid the excess water and the excreta to adapt for the freshwater habitat with lower osmotic pressure. In addition, the presence of podocytes in the Nebenhole in *A. rubens* is effectively separated it from the remaining pericardial cavity surrounding the heart. This separation strongly suggests that Nebenhole is a specific site of the pericardial cavity which helps in urine formation.

The presence of cilia at the apical surface of the podocyte of both the investigated species shows the activity of moving water and excretory materials outside the pericardial gland towards the kidney where they are scavenged.

The cells of the pericardial gland or the podocytes possess a highly developed endocytotic lysosomal digestive system. These cells contain the prominent Golgi complexes with associated vesicles and large membrane-bound electron-dense granules. The vesicles may possibly be the primary lysosomes containing hydrolytic enzymes, which was detected as acid hydrolases by Moore et al. (1980). The electron-dense granules may be the secondary lysosomes or the residual bodies containing materials from intracellular digestive processes. Andrews and Jennings (1993) injected horseradish peroxidase into the blood of *Scorbicularia* sp. and observed that it escaped into primary urine, endocytosed in coated vesicles that accumulated in the lysosomes and residual bodies and finally exocytosed by apocrine secretion.

The basal region of each podocyte is composed of complex cytoplasmic processes or pedicels which give evidences of extensive endocytotic activity. This region, which shows that is specialized for the uptake of extracellular material in the haemocoel. The presence of large endocytotic vesicles and urinary spaces near the base followed by smaller ones in

the center of the podocyte resulted from lysosomal activity gives a reasonable explanation of the existence of pedicels and the more complex cytoplasmic bridges of the podocyte in *A. rubens*. This is concordance with the more need of osmoregulation in the freshwater species than the marine ones.

The extensive lysosomal vacuolar system and the complex system of fenestrated basal membranous folds the investigated species are similar to those found in the visceral epithelial cells of the Bowman's capsule in the mammalian kidney and the podocytes of other vertebrates (Caulfeid & Farquhar, 1976; Kanwar & Farquhar, 1979). This similarity suggests that the basal lamina of the podocyte may play a significant role in the ultrafiltration process and selective filtration mechanism.

The presence of the urinary spaces and intercellular spaces between the podocytes shows that they are the pathways of filtration. The primary urine may be ultrafiltered from the auricular blood spaces through the basal lamina, through the filtration slits of the pedicels and urinary spaces to the intercellular spaces, and then immediately pass into the pericardial cavity. The excretory products may be lysosomed in the vacuolar system inside the podocyte, and then excreted by exocytosis and apocrine secretion to the pericardial cavity. The excreted materials may be driven by cilia of the podocytes out of the pericardial gland through the renopericardial duct, and then to the kidney where it is scavenged by the ciliary movement to the renal duct.

The present ultrastructural investigations show that the suitability of the podocyte structure to its function. For example in *A. rubens*, the wavy apical ciliated surface of the podocyte increases the area of filtration and the surface extensions increase the ability of exocytosis, while in *P. radiata* coated pits increase endocytosis. The presence of extensive Golgi apparatus, endoplasmic reticulum, and the lysosomal vacuolar system in the podocytes of the two species indicate their higher functional activity. The larger number of mitochondria in the podocytes of *P. radiata* than those of *A. rubens* may support the more activity of filtration. Also the presence of the apical desmosomes between the pericardial cells in *P. radiata* give more rigid connection between the podocytes. The more complex interdigitate basal membrane forming pedicles in *A. rubens* increases the endocytotic surface to larger amounts of extracellular materials in the freshwater medium.

References

Andrews, E.B. & Jennlugs, K.H. 1993. The anatomical and ultrastructural basis of primary urine formation in bivalve molluscs. J. Moll. Stud., 95: 233–257.

Caulfield, J.P. & Farquhar, M.G. 1976. Distribution of an ionic sites in glomerular basement membranes, their possible role in filtration and attachment. Proc. Nat. Acad. Sci. U.S.A., 73: 1646–1650.

Cockroft, A.C. 1990. Nitrogen excretion by the surf zone bivalves *Donex serra* and *D. sordidus*. Mar. Ecol. Prog. Ser., 60(1–2): 57–65.

Deaton, L.E. 1992. Osmoregulation and epithelial permeability in two eurhaline bivalve molluscs: *Mya arenaria* and *Geukensia demissa*. J. Exp. Mar. Biol. Ecol., 158(2): 167–177.

Deaton, L.E., Derby, J.G. Subbedar, N. & Greenberg, M.J. 1989.Osmoregulation and salinity tolerance in two species of bivalve molluscs: *Limnoperan fortunei* and *Mytilopsis leucophaeta*. J. Exp. Mar. Biol. Ecol., 133(1–2): 67–79.

Hemelraad, J., Herwig, H.J., Van Donslaar, E.G., Holwerda, D.A. & Zandee, D.I. 1990. Effects of cadmium in freshwater clams. 2. Ultrastructural changes in the renal system of *Anodonta cygnea*. Arach. Environ. Centam. Toxical., 19(5): 691–698.

Jenning, K.H. 1984. The organization, fine structure of the excretory systems of the estuarine bivalve *Scorbicularia plane* (da Costa) and the freshwater bivalve *Anodonta cygnea* (Linne) and other selected

species. Ph.D. Thesis. University of London.

Kanwar, Y.S. & Farquhar, M.G. 1979. Anionic sites in the glomerular basement membrane. In vivo and in vitro localization to the laminae rarae by cationic probes. J. Cell Biol., 81 : 137–153.

Kato, K. 1960. Excretion of the Kebers organ in some lamellibranchs. Sci. Rep. Saitama Univ. (B0), 3: 233–244.

Moore, N., Bubel, A. & Lowe, D.M. 1980. Cytology and cytochemistry of the pericardial gland cells of *Mytilus edulis* and their lysosomal responses to injected horseradish peroxidase and anthracene. J. Mar. Biol. Ass. U.K., 60: 135–149.

Morse, M.P. 1990. Experimental demonstration of a 3-D muscle network in the pericardial gland of the bivalve mollusc, *Chlamys hastata* and its possible role in ultrafiltration. Am. Zool., 30 (46A).

Narian, A.S. 1976. A review of the structure of the heart of molluscs, particularly bivalves, in relation to cardiac function. J. Moll. Stud., 42: 46–62.

Peggs, D. 1975. Aspects of excretory ultrafiltration in *Anodonta cygnea* (L). M.Sc. Thesis, University of Manchester.

Pirie, B.J.S. & George, S.G. 1979. Ultrastructure of the heart and excretory system of *Mytilus edulis* (L.). J. Mar. Biol. Ass. U.K., 59: 819–829.

Potts, W.T.W. 1954. The rate of urine production of *Anodonta cygnea.* J. Exp. Biol., 31: 614–617.

Potts, W.T.W. 1967. Excretion in the molluscs. Biol. Rev., 42: 1–14.

White, K.M. 1942. The pericardial cavity and the pericardial gland of the Lamellibranchia. Proc. Malacol. Soc. Lond., 52: 37–88.

Wohlgeschaffen, G.D., Mann, K.H., Subb Rao, D.V. & Pocklington, R. 1992. Dynamics of the phycotoxin domic acid: Accumulation and excretion in two commercially important bivalves. J. Appl. Phycol; 4(4): 297–310.

Explanation of plates I-V

Ultrastructure of podocyte in *Pinctada radiata*

Plate I

Fig. 1. The cell bodies showing cytoplasmic organelles, scale bar = 0.3 μm.
Fig. 2. The apical surface of podocytes, scale bar = 0.5 μm.
Fig. 3. The apical part of adjacent podocytes showing desmosome junction, coated pits and the structure of cilia, scale bar = 1 μm.
Fig. 4. The cell bodies showing endocytotic vesicles, intercellular spaces between cells and the distribution of chromatin granules in the nuclei, scale bar = 0.5 μm.

Plate II

Fig. 5. The cell body showing the nucleus, nucleolus, and smooth endoplasmic reticulum, scale bar = 1 μm.
Fig. 6. The cell body showing electron-dense granules in various shapes and sizes, scale bar = 1 μm.
Fig. 7. The cell body showing some cytoplasmic organelles, scale bar = 0.75 μm.
Fig. 8. The cell body showing the units of endoplasmic reticulum and Golgi apparatus. Note the close association of them with primary lysosomes., scale bar = 1 μm.
Fig. 9. The basal region of the podocyte showing the pedicels and urinary spaces, scale bar = 0.75 μm.

Plate III

Fig. 10. The basal part of the cell shows large endocytotic vesicles and its inclusions, 1. Electron-dense granule, 2. Electron lucent, 3. dark granules, scale bar = 1 μm.
Fig. 11. The fenestrated basal membrane of the cell showing pedicels, urinary spaces and muscle fibers, scale bar = 1 μm.

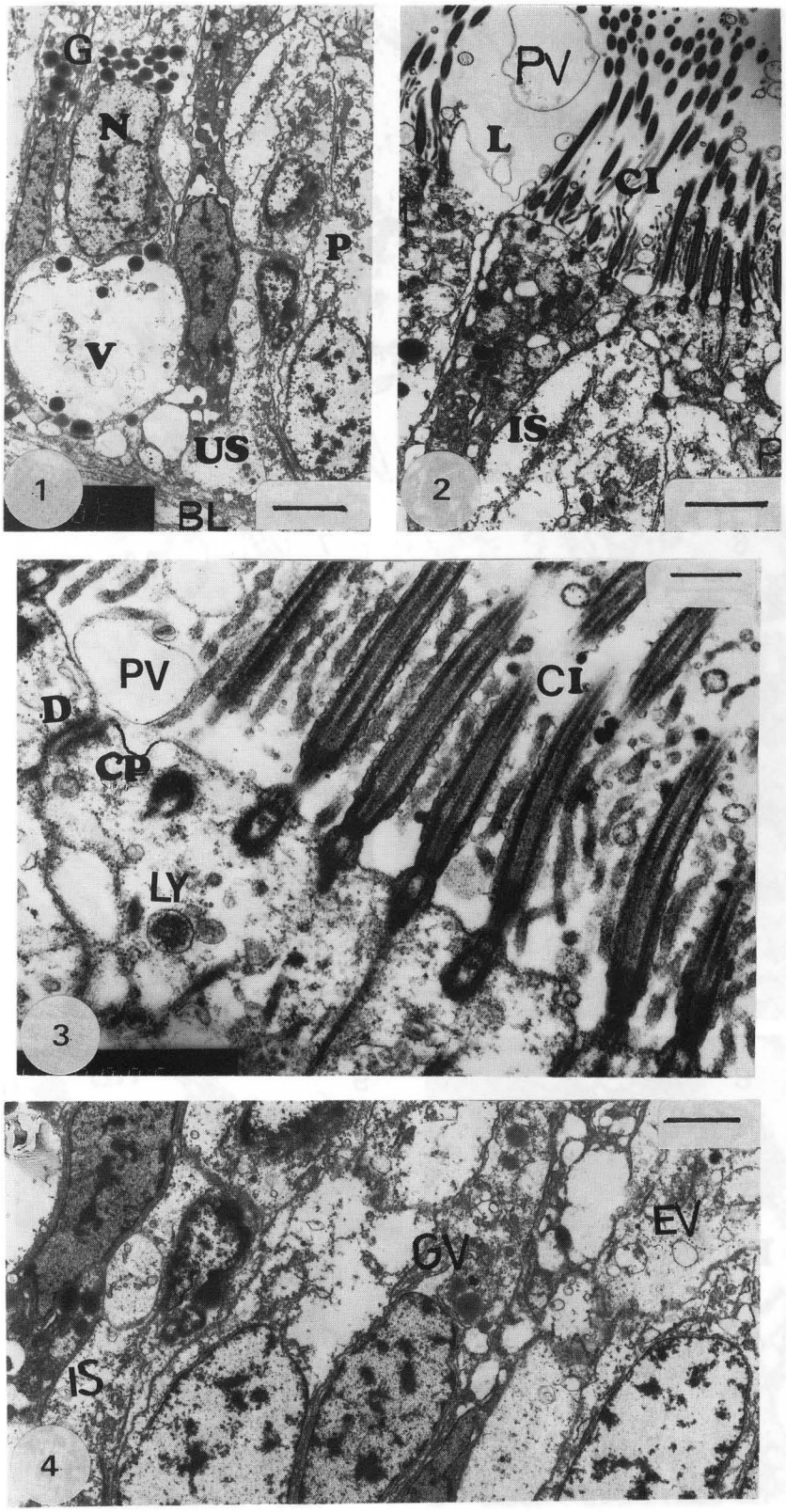
Ultrastructure of podocyte in *Anodonta rubens*

Plate IV

Fig. 1. The cell wall of the pericardial gland. Note the shape of podocytes with numerous cilia, scale bar = 0.3 μm.
Fig. 2. The cell bodies showing their cilia and apocrine papillae, scale bar = 0.5 μm.

Plate V

Fig. 3. The cytoplasmic granules, scale bar = 0.75 μm.
Fig. 4. The cell bodies showing intercellular spaces, scale bar = 0.5 μm.
Fig. 5. The cell body showing endocytotic vesicles with its inclusions, lysosomal vascular system, Golgi apparatus, mitochondria and nucleus, scale bar = 1 μm.
Fig. 6. The basal membrane of the cell showing pedicels and urinary spaces, scale bar = 0.75 μm.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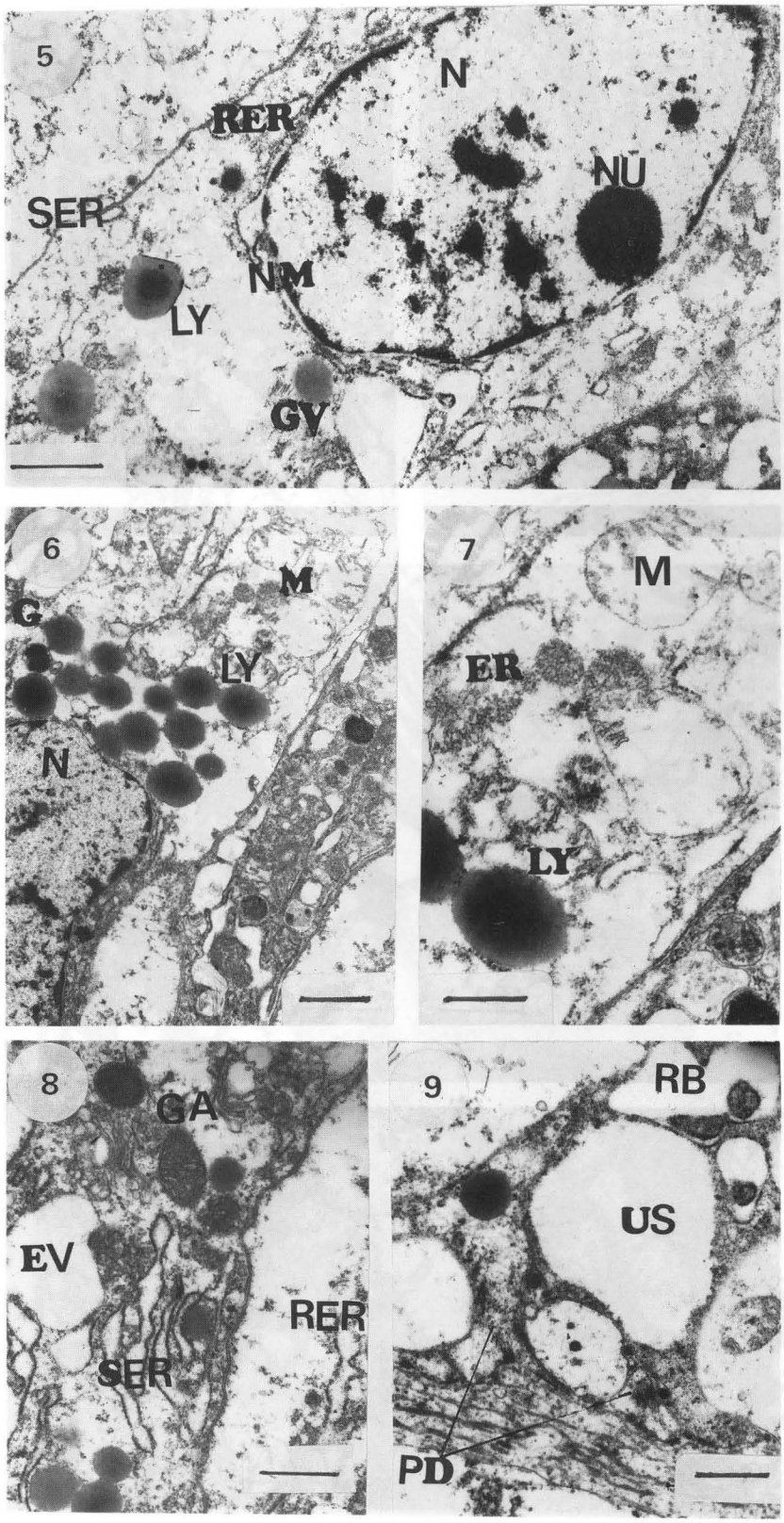
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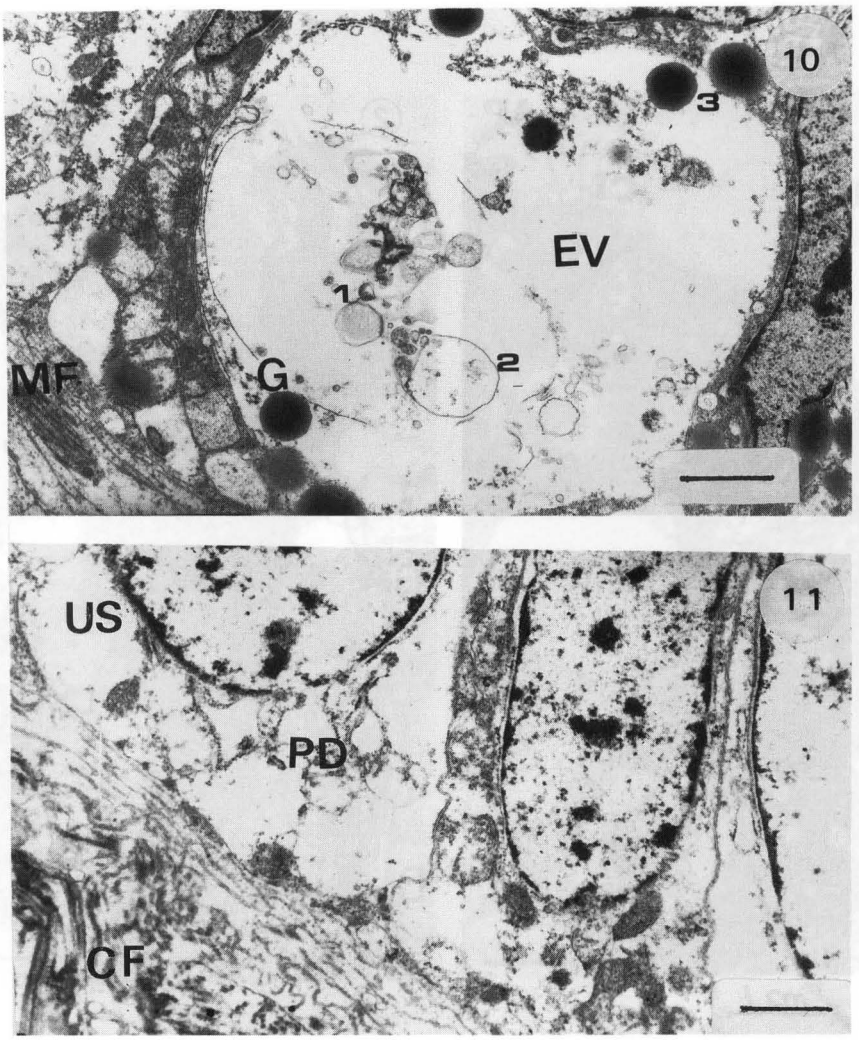
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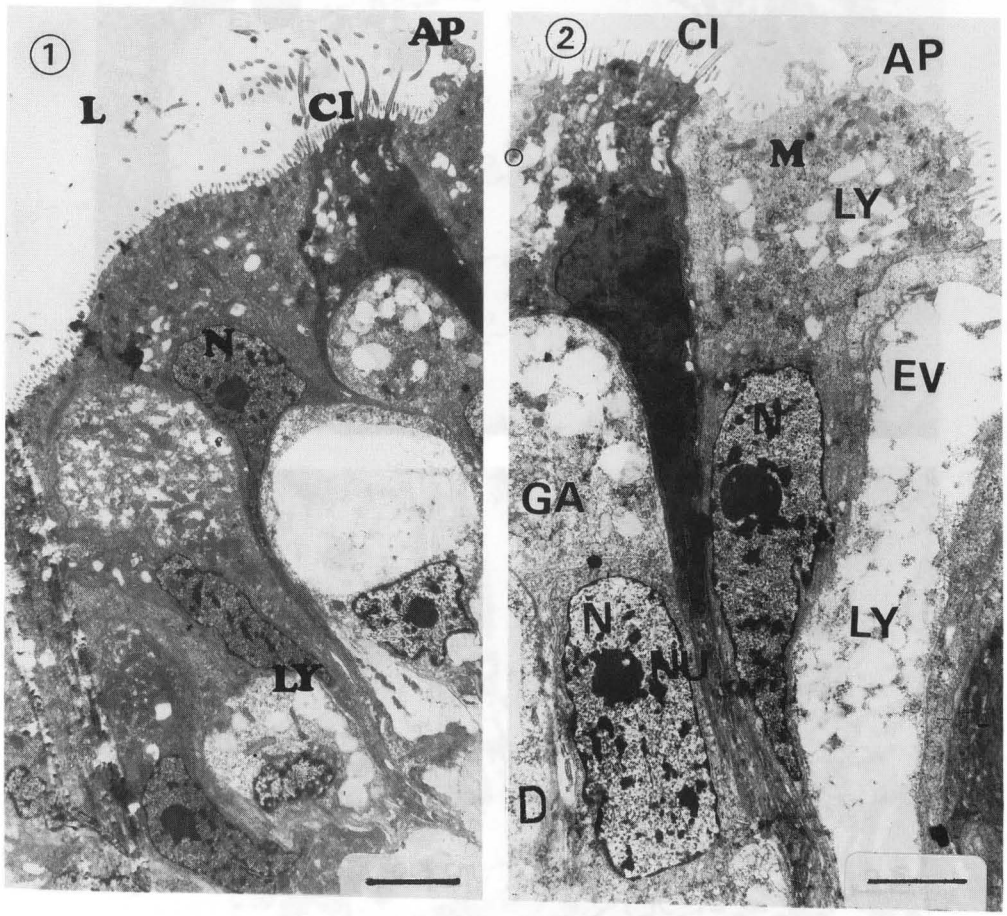
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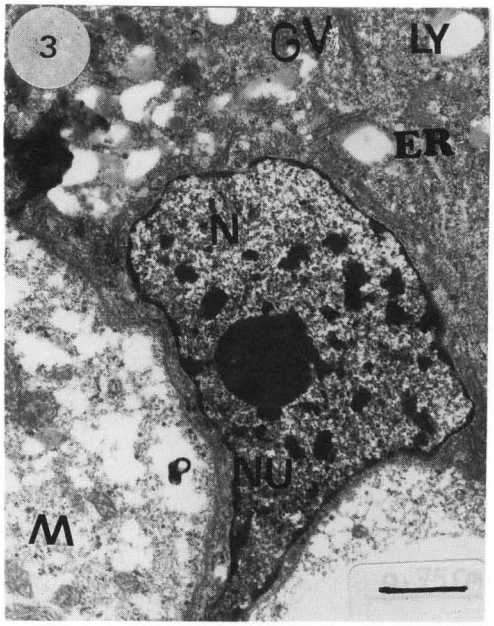
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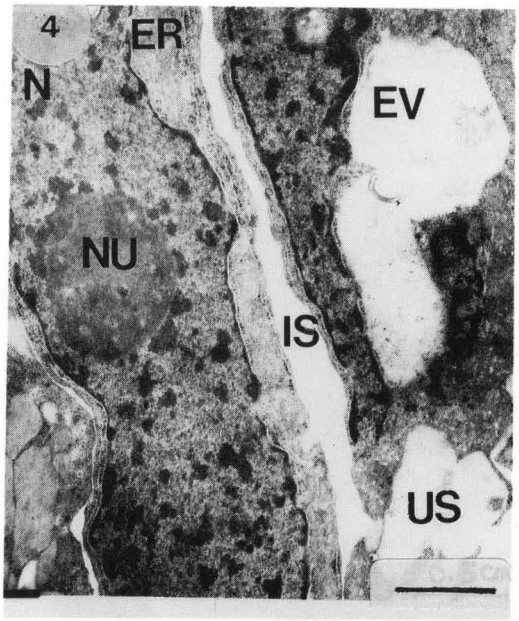
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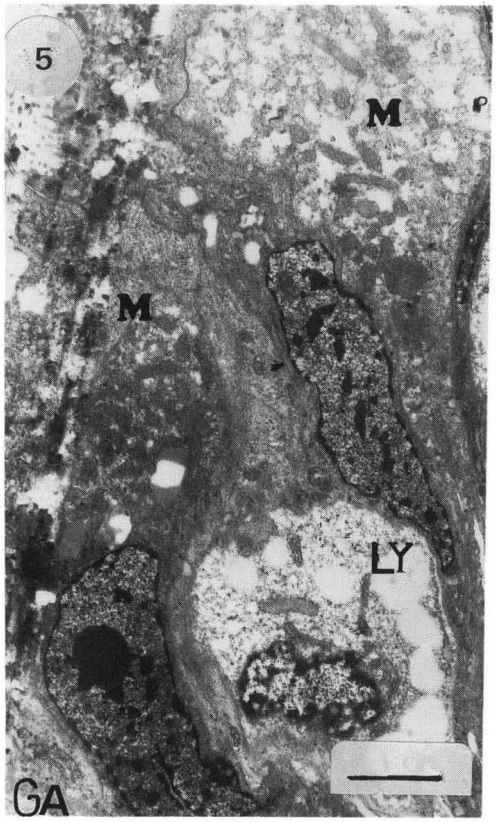
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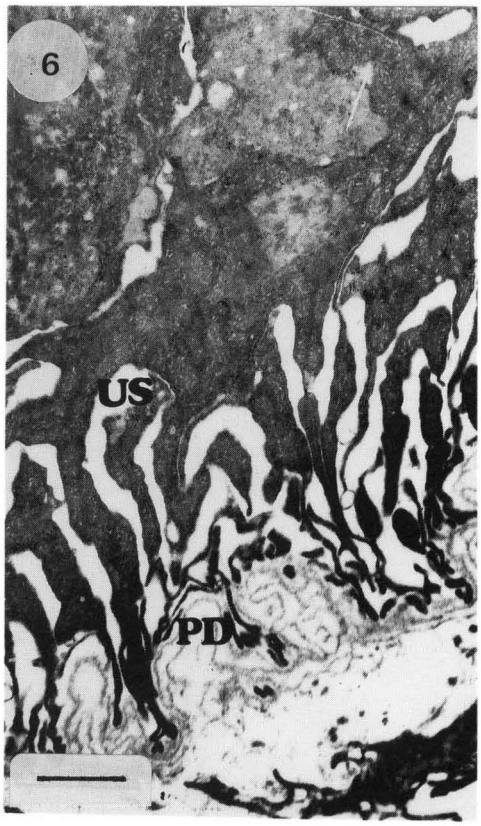
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